

APPLICATION NOTE

DETERMINING THE AMOUNT OF BLUE DYE PRESENT IN FOUR PEANUT CHOCOLATE CANDIES USING UV-VIS ABSORPTION SPECTROSCOPY



INTRODUCTION

Brilliant Blue (also known as Food Blue 2, E133, Blue No.1, or Brilliant Blue FCF) is a synthetic non-fluorescent and water-soluble dye with a reddish-blue appearance that is widely used in the preparation of foods, pharmaceuticals, and cosmetics items (the estimated average consumption is around 16 mg per person per day), see **Figure 2** [1-3].

As a very stable blue color dye that tends to fade at pH values higher than 8, Brilliant Blue is commonly used in combination with Tartrazine (E102) or Quinoline Yellow (E104) coloring agents to give green shades and with other coloring agents to give brown or black shades [1-3].

In addition to applications in foods, pharmaceuticals, and cosmetics items, Brilliant Blue is also used in the identification of water distribution in soil [3,4].

Keywords:

UV-Visible Absorption Spectroscopy
 DWHP Light Source
 FLEX Spectrometer
 Brilliant Blue FCF
 Peanut Chocolate Candies



Figure 1 – Absorbance Configuration.



In this application note, we combine the DWHP light source with FLEX spectrometer in an absorbance configuration to determine the amount of blue dye present in four different blue peanut chocolate candies.

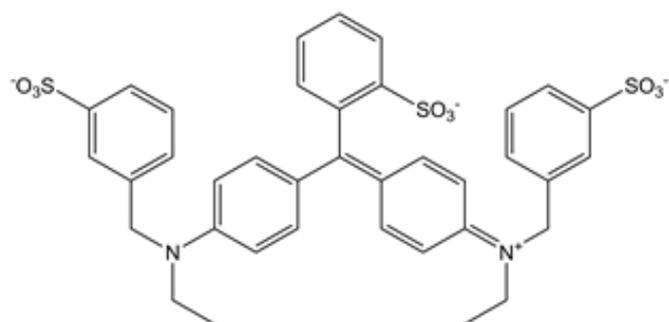


Figure 2 – Chemical structure of Brilliant Blue FCF.

MATERIALS & METHODS

Reagents

- Blue peanut chocolate candies;
- Distilled Water;
- Brilliant Blue FCF;

Instruments and Accessories:

Absorbance Configuration

(see **Figure 1**)

- DWHP light source;
- 200 μm diameter illumination optical fiber;
- Standard cuvette holder set into an absorbance configuration;
- 200 μm diameter collecting optical fiber;
- FLEX STD UV/Vis spectrometer (Slit: 10 μm);
- 10x10 mm absorption cuvettes in UV quartz;

Table 1 – Instrument settings used for experimental diffuse reflectance measurements.

Parameter	Used Settings
Integration time (ms)	5
Average	100
Smoothing	2

EXPERIMENTAL PROCEDURE

1. Insert a blue peanut chocolate candy in a 20 mL volumetric flask and add 7 mL of distilled water. Be sure the chocolate candy is completely covered with water.
2. Once all of the dye has been dissolved in the solution A (the peanut chocolate candy loses the blue color and becomes white), remove the candy. At this point, it might be necessary to wait around 10 minutes for large particles to precipitate.
3. Then, carefully take 1 mL from the top of Solution A and dilute by adding 10 mL of distilled water (Solution B).
4. Take the 3 mL from solution B that must be completely homogeneous and measure the absorption spectra between 250 and 750 nm, using filtered distilled water as a reference. The solution in the flask should be colored but allow light to clearly pass through.
5. The LightScan software was used with the instrument's settings specified in **Table 1**.

RESULTS

The absorption spectra of both Brilliant Blue FCF and blue dye solution are presented in **Figure 3**.

The absorption spectrum of Brilliant Blue FCF is very similar to that obtained for the blue dye extracted from the peanut chocolate candy with both spectra exhibiting similar peaks at around 312, 413, and 632 nm.

The difference observed between the Brilliant Blue FCF and blue dye spectra is the baseline of the latter, which continuously increases as the wavelength decreases. It may come from the presence of other compounds in the peanut chocolate candy that are dissolved with the blue dye and absorb in that region.



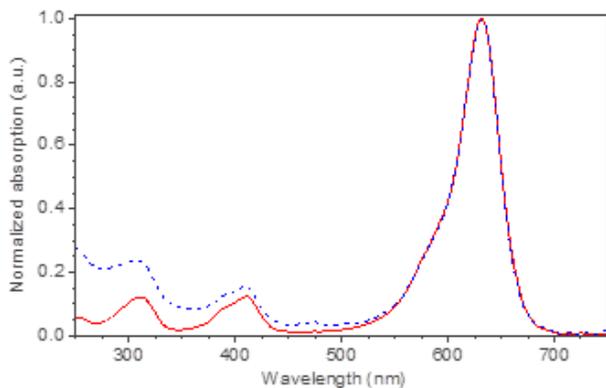


Figure 3 – Normalized absorption spectra of Brilliant Blue FCF (red line) and blue peanut chocolate candy (blue dashed line) solutions.

In order to determine the amount of blue dye present in four peanut chocolate candies, it was necessary to perform a calibration curve using the Brilliant Blue FCF to estimate its molar absorption coefficient. The calibration curve obtained for different solutions of Brilliant Blue FCF is presented in **Figure 4**.

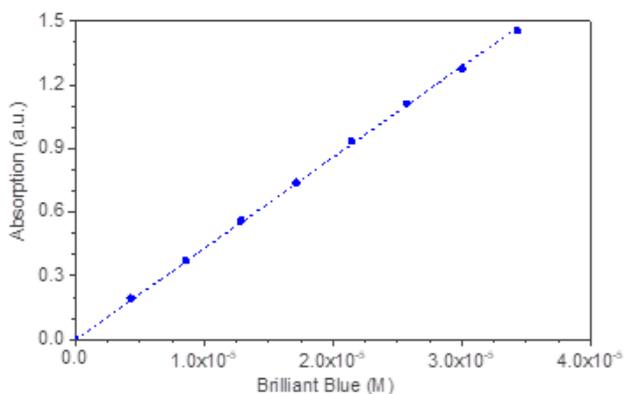


Figure 4 – Calibration curve ($y=42887x$ with $R^2=0.999$) obtained from the absorption of Brilliant Blue FCF at 632 nm for molar absorption coefficient calculation.

By taking the molar absorption coefficient at 632 nm, where other compounds from the peanut chocolate candies that are solubilized by water do not affect the absorbance value at the peak maximum, we can determine the amount of blue dye present in peanut chocolate candies.

The amount of blue dye present in four different peanut chocolate candies is given in **Table 2**.

Table 2 – Amount of blue dye present in four different chocolate candies (CC).

PCC	PCC Initial Weight ^a (g)	PCC final Weight ^a (g)	Dye Conc. (M)	Dye Weight (mg)
1	1.846	1.537	1.6×10^{-4}	0.882
2	3.077	2.691	1.7×10^{-4}	0.952
3	2.752	2.422	1.9×10^{-4}	1.03
4	1.379	1.113	1.2×10^{-4}	0.668

^a PCC total weight before adding water. b after adding water and remove the colored cover from the PCC.

As expected, the amount of dye depends on the size and weight of the peanut chocolate candy, being normally lower than 1 mg per candy, and not higher than 0.06% of the peanut chocolate candy total weight. Some exceptions might be expected due to the immense variety of candies in size and weight.

The blue dye used in peanuts chocolate candies as coloring agents, as with most of the synthetic coloring agents used in food, has a low acute toxicity. The EFSA panel established a new acceptable daily intake to Brilliant Blue FCF equal to 6 mg per kg of bodyweight per day [5].

CONCLUSIONS

The amount of blue coloring agent present in four peanut chocolate candies was estimated using a calibration curve with Brilliant Blue FCF, which is the same coloring agent used to color the peanuts chocolate candies. For a candy with total weight of 3 grams, it was estimated that the amount of dye is around 1 mg, which corresponds approximately to 0.04% of the total weight of the candy.

With this application note, we want to demonstrate that combining DWHP light source, FLEX spectrometer, and all the accessories required to assemble the absorption configuration allows for easy, fast, reliable, and accurate measurements in food coloring agents quantification.



REFERENCES

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